NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 419

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

HC YELLOW 4

(CAS NO. 59820-43-8)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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NTP TECHNICAL REPORT

ON THE

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P.O. Box 12233
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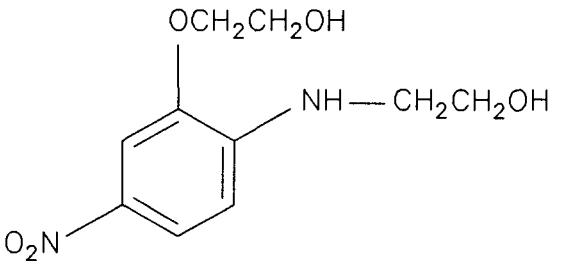
ABSTRACT



HC YELLOW 4

CAS No. 59820-43-8

Chemical Formula: $C_{10}H_{14}N_2O_5$ Molecular Weight: 242.2

Synonym: N,O-di(2-hydroxyethyl)-2-amino-5-nitrophenol

HC Yellow 4 is used in semipermanent hair dyes. Toxicology and carcinogenesis studies were conducted by administering HC Yellow 4 (greater than 93% pure) in feed to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, and *Drosophila melanogaster*.

14-Day Studies: Groups of five rats of each sex were given 0, 5,000, 10,000, 20,000, 40,000, or 80,000 ppm and groups of five mice of each sex were given 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm HC Yellow 4 in feed for 14 days. All animals survived to the end of the studies. Final mean body weights of male rats that received 20,000 ppm or more, female rats that received 10,000 ppm or more, and female mice that received 20,000 ppm were significantly lower than those of the controls. The mean body weights of exposed and control groups of male mice were similar. No chemical-related decrease in feed consumption was observed. No chemical-related clinical findings or changes in absolute or relative organ weights occurred in rats or mice. No gross or microscopic changes were related to HC Yellow 4 administration in rats or mice.

13-Week Studies: Groups of 10 rats of each sex were fed diets containing 0, 2,500, 5,000, 10,000, 20,000, or 40,000 ppm and groups of 10 mice of each sex were fed diets containing 0, 5,000, 10,000, 20,000, 40,000, or 80,000 ppm HC Yellow 4 for 13 weeks. All rats survived to study termination. Chemical-related deaths occurred at the two highest dose levels in male and female mice. Final mean body weights of male rats that received 10,000 ppm or greater, female rats that received 20,000 or 40,000 ppm, and mice that received 10,000 ppm or greater were significantly lower than those of the controls. There were no biologically significant changes in absolute or relative organ weights. Mineralization of the renal papilla occurred in all male rats in the 40,000 ppm group. Thyroid pigmentation occurred in rats receiving 40,000 ppm and in mice at all dose levels. Uterine atrophy occurred in female rats in the 20,000 and 40,000 ppm groups and female mice in the 40,000 and 80,000 ppm groups. Lymphoid depletion and atrophy of the spleen occurred in male mice that received 40,000 or 80,000 ppm and female mice that received 80,000 ppm. Atrophy of the thymus occurred in male and female mice that received 40,000 or 80,000 ppm.

2-Year Studies: Groups of 70 male rats were fed diets containing 0, 2,500, or 5,000 ppm and groups of 70 female rats and 70 mice of each sex were fed diets containing 0, 5,000, or 10,000 ppm HC Yellow 4 for up to 2 years. Interim evaluations were performed on 10 rats and 10 mice from each dose group at 6 and 15 months. No biologically significant changes in absolute or relative organ weight or hematology or clinical chemistry values were found in these rats or mice. No compound-related lesions were seen in exposed rats. In exposed mice, pigmentation of the thyroid gland was observed at the 6-month interim evaluations; pigmentation and hyperplasia of the thyroid gland were seen at the 15-month interim evaluations.

Body Weight, Survival, and Feed Consumption in the 2-Year Studies: The mean body weight of female rats that received 10,000 ppm was significantly lower than that of the controls. The mean body weights of mice receiving 10,000 ppm were 20% to 30% lower than those of the controls during the second year of the studies. The survival of exposed rats and mice was similar to that of the controls.

Neoplasms and Nonneoplastic Lesions in the 2-Year Studies: Pituitary gland pars distalis adenomas were marginally increased in exposed male rats (0 ppm, 17/45; 2,500 ppm, 20/49; 5,000 ppm, 28/49), and there was a concomitant dose-related increase in the incidence of hyperplasia (8/45, 13/49, 18/49). There was no increase in the incidence of pituitary gland adenomas or carcinomas in female rats (34/49, 35/48, 30/49).

In mice, no neoplasms were considered related to chemical administration. However, a dose-related increased incidence of thyroid gland pigmentation and follicular cell hyperplasia occurred in both sexes of mice.

Genetic Toxicology: HC Yellow 4 was mutagenic in *Salmonella typhimurium* strains TA100, TA1537, and TA98 with and without exogenous metabolic activation (S9); the response in strain TA1535 without S9 was equivocal. HC Yellow 4 induced sister chromatid exchanges in Chinese hamster ovary cells in the absence but not the presence of S9 activation; no induction of chromosomal aberrations occurred in Chinese hamster ovary cells, with or without S9. HC Yellow 4 induced sex-linked recessive lethal mutations in germ cells of adult male *Drosophila melanogaster* when administered by injection; results of a reciprocal translocation test in *D. melanogaster* were negative.

Conclusions: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity** of HC Yellow 4 in male F344/N rats based on the increased incidence of pituitary gland adenomas and hyperplasia. The male rats may have been able to tolerate a slightly higher dose of the chemical. There was *no evidence of carcinogenic activity* of HC Yellow 4 in female F344/N rats given 5,000 or 10,000 ppm. There was *no evidence of carcinogenic activity* of HC Yellow 4 in male or female $B6C3F_1$ mice given 5,000 or 10,000 ppm.

There was a chemical-related increase in the incidence of thyroid gland pigmentation and follicular cell hyperplasia in mice.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 10.

Summary of the 2-Year Carcinogenicity and Genetic Toxicology Studies of HC Yellow 4

Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Doses			
0, 2,500, or 5,000 ppm in feed	0, 5,000, or 10,000 ppm in feed	0, 5,000, or 10,000 ppm in feed	0, 5,000, or 10,000 ppm in feed
Body weights			
Dosed groups similar to controls	High-dose group lower than controls	Dosed groups lower than controls	Dosed groups lower than controls
2-Year survival rates			
21/50, 29/50, 28/50	27/50, 31/50, 34/50	28/50, 29/50, 35/50	43/50, 38/50, 43/50
Nonneoplastic effects			
None	None	Thyroid gland: follicular cell pigmentation (0/47, 44/48, 49/49), follicular cell hyperplasia (0/47, 27/48, 41/49)	Thyroid gland: follicular cell pigmentation (0/48, 49/49, 50/50), follicular cell hyperplasia (0/48, 3/49, 13/50)
Neoplastic effects			
None	None	None	None
Uncertain findings			
Pituitary gland pars distalis: adenoma (17/45, 20/49, 28/49), hyperplasia (8/45, 13/49, 18/49)	None	None	None
Level of evidence of carcinogenic activity			
Equivocal evidence	No evidence	No evidence	No evidence
Genetic toxicology			
Salmonella typhimurium gene mutation:	Positive with and without S9 in strains TA100, TA1537, and TA98; equivocal without S9 in strain TA1535		
Sister chromatid exchanges			
Chinese hamster ovary cells *in vitro*:	Negative with S9, positive without S9		
Chromosomal aberrations			
Chinese hamster ovary cells *in vitro*:	Negative with and without S9		
Sex-linked recessive lethal mutations			
Drosophila melanogaster:	Positive by injection; negative by feeding		
Reciprocal translations			
Drosophila melanogaster:	Negative		

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on HC Yellow 4, NTP TR 419 on 9 July 1991 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 9 July 1991, the draft Technical Report on the toxicology and carcinogenesis studies of HC Yellow 4 received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of HC Yellow 4 by discussing the uses, describing the experimental design, reporting on survival and body weight effects, and commenting on neoplastic and nonneoplastic lesions in male rats and in mice. The proposed conclusions were *equivocal evidence of carcinogenic activity* of HC Yellow 4 in male rats and *no evidence of carcinogenic activity* of HC Yellow 4 in female rats and male and female mice.

Dr. Zeise, a principal reviewer, agreed in principle with the proposed conclusions. She thought the conclusions should note that male and female rats could have tolerated significantly higher doses. Dr. Zeise said that the increased incidence of uterine stromal polyps in female rats should be considered "may have been related to chemical administration," unless there are better reasons for discounting them than that the incidence in treated animals falls within the range of overall NTP historical controls. Dr. Dunnick commented that more historical control data would be added and that there were no supporting nonneoplastic effects, providing further evidence that these lesions probably were not chemical related. Dr. J.K. Haseman, NIEHS, added that the rate of uterine polyps in the high-dose group was similar to the historical control mean from previous studies at this laboratory. Further, based on the results of previous NCI/NTP studies, it would be unusual for a chemical to induce only uterine polyps.

Dr. Carlson, the second principal reviewer, agreed with the proposed conclusions.

Dr. Garman, the third principal reviewer, agreed with the proposed conclusions. Because of the prominent chemical-related increased frequency of thyroid follicular cell hyperplasia in the 2-year studies in mice, he thought it appropriate to add frequency figures to the summary table in the Abstract.

Dr. Zeise moved that the Technical Report on HC Yellow 4 be accepted with the revisions discussed and with the conclusions as written, *equivocal evidence of carcinogenic activity* in male rats and *no evidence of carcinogenic activity* in female rats and male and female mice, and with the addition of a statement that "male and female rats may have been able to tolerate higher doses." Dr. Garman seconded the motion. Dr. Goodman offered an amendment that the added statement be removed. Dr. Klaassen seconded the amendment, which was accepted by seven yes to three no votes (Drs. Carlson, McKnight, and Zeise). Dr. McKnight offered an amendment to add a statement to the conclusions that "male rats may have been able to tolerate a higher dose." Dr. Zeise seconded the amendment, which was accepted by seven yes to three no votes (Mr. Beliczky and Drs. Goodman and Hayden). Dr. Zeise's amended motion was then accepted unanimously with ten votes.